In the Claims:

Please amend claims 84, 85, 89, 94, 95, 97, 99 and 101, and add new claim 102, as indicated in Appendix B. A clean copy of the entire set of pending claims is set forth in Appendix A.

## REMARKS

Claims 82, 84, 85, 89, 94-97, 99 and 101 are currently pending. Claims 84, 85, 89, 94, 95, 97, 99, and 101 have been amended, and a marked-up version of the amended claims is submitted herewith as Appendix B. New claim 102 has been added. Thus, after entry of this amendment, claims 82, 84, 85, 89, 94-97, 99, 101, and 102 will be pending. A clean version of the entire set of pending claims is submitted herewith as Appendix A for the Examiner's convenience.

Claims 84, 85, 89, 94, 95, and 97 have been amended to correct obvious typographical errors, and not for reasons related to patentability.

Applicants have added new claim 102 to recite the pharmaceutical composition according to claim 97, wherein the pharmaceutical composition comprises from about $1 \times 10^6$ to $1 \times 10^7$ antigen-activated dendritic cell precursors. No new matter has been added by way of this amendment, and support can be found, *e.g.* at page 41 of the specification.

Applicants acknowledge that the objections to claims 83-85, 89, 94-97 and 99 made in the 03 January 2001 Office Action have since been withdrawn in the Advisory Action dated 08 August 2001.

I. Rejection Under 35 U.S.C. § 112, Second Paragraph

Claim 99 stands rejected under 35 U.S.C. § 112, second paragraph, as allegedly being "ambiguous" for the recitation of "milligrams of antigen per dose." The Examiner opines that "the claim in reciting a finite amount of antigen without giving an indication what a 'dose' of cells comprises gives no indication regarding the ability of the 'dose' of dendritic cells to present a sufficient level of processed peptide on their surface for presentation to reactive T cells" (Paper No. 11, page 3). The Examiner further opines that claim 99 "does not indicate how to divide into doses or how many cells can carry a dose" (Paper No. 15).

Solely in an effort to advance prosecution of this application, claim 99 has been amended to delete the phrase "per dose" and recite instead the pharmaceutical composition according to claim 97, wherein the antigen-activated dendritic cells express an amount of modified antigen to provide between about 1 to 100 micrograms of modified antigen [per dose] in said pharmaceutical composition. No new matter has been added by way of this amendment.

Applicants submit that, at the time the earliest priority date for the instant application, it was well-known to skilled artisans how to elute peptides presented by MHC molecules on the surface of a population of cells (*e.g.*, by acid extraction) and determine the quantities of those peptides (see, *e.g.,* Hunt *et al.* (1992) *Science* 255:1261-1263, paragraph spanning pages 1261-1262, attached hereto as Appendix C). Thus, it would be well within the skill of an artisan to determine the "finite amount" of modified antigen expressed by the antigen-activated dendritic cells in the pharmaceutical composition recited in claim 99.

Thus, Applicants submit that claim 99, as amended, satisfies the requirements of 35 C.F.R. § 112, second paragraph.

Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Claims 83-85, 89, 94, 95 and 101 also stand rejected under 35 U.S.C., second paragraph, as allegedly being indefinite for the recitation of "processed antigen presenting dendritic cell precursors" which present antigen in independent claim 101. The Examiner opines that "once dendritic cells express antigenic fragments on their cell surface, they are no longer precursors, rather they become mature antigen presenting cells." (Paper No. 11, page 4).

Applicants respectfully traverse this ground of rejection.

Respectfully, the Examiner has provided no evidence whatsoever to support this contention. Throughout the specification, Applicants have provided ample evidence that dendritic cell precursors are capable of processing and presenting antigen. Applicants respectfully draw the Examiner's attention, *e.g.,* to pages 35-36 of the specification. Applicants state that

> Mature dendritic cells, while effective in sensitizing T cells to several different antigens, show little or no phagocytic activity. To the extent that endocytosis is required for antigen processing and presentation, it was not previously evident how dendritic cells would present particle-associated peptides. Based on our work, it is now evident that progenitors to dendritic cells which this invention provides can internalize such particles for processing and presentation....Phagocytosis of particulate matter by dendritic cell precursors may be accomplished by culturing the dendritic cell precursors in the presence of particulate matter for a time sufficient to allow the cells to phagocytose, process and present the antigen (specification, page 35, line 34 – page 36, line 20).

The Applicants also state that

> The phagocytic dendritic precursor cells are obtained by stimulating cell cultures comprising dendritic precursor cells with GM-CSF to induce aggregates of growing dendritic cells...If the developing cultures are

> exposed to particles, washed and "chased" for 2 days, the number of MHC-class II rich dendritic cells increases substantially and at least 50% contain internalized particles such as BCG mycobacteria or latex particles. The mycobacteria-laden, newly developed, dendritic cells are much more potent in presenting antigens to primed T cells than corresponding cultures of mature dendritic cells that are exposed or pulsed with antigen (specification, page 37, lines 4-19; emphasis added).

Thus, Applicants have successfully shown that precursor dendritic cells are better at processing antigens for presentation than their mature dendritic cell counterparts.

The Examiner is further directed to Figure 12, which shows Diff-Quick stains of developing dendritic cells that have been exposed to latex and carbon, in an effort to look at phagocytosis capability of precursor dendritic cells as compared to mature dendritic cells. Figure 12A shows that an aggregate of developing dendritic cells, which were cytospun after 20 hours of exposure to 2μ latex spheres, are labeled with the uniform latex particles (see, *e.g.,* page 16, and Example 3H, page 68). The cells in this aggregate correspond to precursor dendritic cells, which are capable of maturation and are eventually released as nonproliferating, mature dendritic cell progeny (see, *e.g.,* pages 54-56). Figure 12B shows the same as Figure 12A, except that the cultures were chased for a day to allow the production of mature single dendritic cells, which were released into suspension. Many of the released, mature dendritic cells derived from the proliferating dendritic cell precursors also contain the uniform latex particles (see, *e.g.,* page 16, and Example 3H, page 68). Finally, Figure 12D shows that when mature dendritic cells were exposed to carbon after they had been produced from proliferating aggregates of precursor dendritic cells, carbon deposits were not evident (see, *e.g.,* page 16, and Example 3H, page 68).

Applicants have further shown in Figure 15 that immature dendritic cell precursors, which have been pulsed with antigen, are capable of presenting antigen to T cells (see also

Example 3K, page 71). In Figure 15A, for instance, a comparison is made, *inter alia*, between (a.) immature dendritic cell precursors pulsed for 1 day with BCG antigen (▼); (b.) immature dendritic cell precursors pulsed for 1 day with BCG antigen and allowed to mature (chased for 2 days) (♦); and (c.) mature dendritic cells pulsed with BCG antigen (▲). While the pulse-chased cells presenting BCG were most potent, it is clear that the immature dendritic cell precursors, which were tested immediately after the 1 day pulse, also elicited a sizeable T cell response to mycobacterial antigen. In contrast, the mature dendritic cells that had been pulsed were less capable of eliciting T cell responses. These experiments show that the extent of phagocytosis in each cell population (discussed above) directly corresponds with antigen presentation and T cell activation capabilities (which is further evidenced in Table 1, page 70).

Taken together, the Applicants have discovered, and their experimental evidence indicates, that both precursor dendritic cells pulsed with antigen, as well as mature dendritic cells that arise from precursor dendritic cells pulsed with antigen, can process and present antigens; whereas mature, non-proliferating dendritic cells pulsed with antigen cannot.

The results seen herein are similar to those seen for dendritic cells isolated from the epidermis. Freshly isolated epidermal Langerhans cells, are immature, but non-proliferating dendritic cells (see specification, *e.g.,* at page 7, line 24). Immature epidermal Langerhans cells have been similarly shown to have the ability to process and present antigen to T cells, while mature Langerhans cells do not have that ability. See, *e.g.,* Abstract of Romani *et al.* (1989) *J. Exp. Med.* 169:1169-1178, cited previously on form PTO-1449, but is submitted herewith as Appendix D for the Examiner's convenience. However, neither report teaches or suggests the

occurrence of phagocytosis of antigens contacted with cultures of proliferating dendritic cell precursors, as has been discovered by the Applicants.

Solely in an effort to advance prosecution of this application, Applicants have amended claim 101 to delete the "processed antigen presenting dendritic cell precursors" language and recite instead an *in vitro* composition comprising a population of antigen-activated dendritic cell precursors, wherein said antigen-activated dendritic cell precursors present processed antigen derived from an enriched and expanded population of proliferating dendritic cell precursors, which were contacted *in vitro*, in the presence of GM-CSF, with antigen for sufficient time for said proliferating dendritic cell precursors to process and present said antigen. No new matter has been added by way of this amendment, and support can be found throughout the specification, *e.g.*, at pages 41-42.

Applicants submit that claim 101, as amended, satisfies the requirements of 35 C.F.R. § 112, second paragraph. Likewise, claims 84, 85, 89, 94, and 95 (and new claim 102), which are directly or indirectly dependant on claim 101, as amended, and thus contain all the limitations thereof, also satisfy the requirements of 35 C.F.R. § 112, second paragraph.

Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

II. Rejection Under 35 U.S.C. § 102.

Claims 82-85, 89, 94-97 and 99 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Markowicz *et al.* (J. Clin. Invest. 85:955-961). The Examiner opines that

Markowicz *et al.* teaches "the isolation and maturation of DCs from human peripheral blood by the in vitro addition of GM-CSF," and, thus, anticipates the claimed invention.

Applicants respectfully traverse this ground of rejection.

With respect to the rejection of claims 82-85, 95-97, and 99, Markowicz *et al.* fails to disclose or suggest an enriched and expanded population of proliferating dendritic cell precursors. Instead, Markowicz *et al.* teaches that GM-CSF affects the morphology and viability of dendritic cells isolated from peripheral blood, as well as the survival and differentiation of non-proliferating dendritic cells. For instance, Markowicz *et al.* states:

> As shown in Fig. 4, the number of differentiated (branched DC) increased as the concentration of GM-CSF in the culture increased. At any given concentration of the cytokine, however, the total number of viable cells as well as the number of branched cells per well remained stable over time suggesting that GM-CSF does not cause DCs to divide and proliferate (Markowicz *et al.,* page 958; emphasis added).

Thus, Markowicz *et al.* teaches away from enriched and expanded population of proliferating dendritic cell precursors. Moreover, the data presented in Figure 4 also indicates that the dendritic cell number is kept constant over time from day 11 to day 24. A skilled artisan would understand that if the population of dendritic cells were expanded and proliferating, as claimed by the Applicants, this number would increase over time. This observation in Markowicz *et al.* is in contrast to the observations made by the Applicants, which clearly demonstrates the expansion of dendritic cell cultures from proliferation dendritic cell precursors.

> In summary, from a starting blood mononuclear culture of $1.5 \times 10^6$ cells, where dendritic cells were difficult to detect, we on average obtained 5-10 subcultures each with at least $3\text{-}10 \times 10^4$ released dendritic cells at 3 weeks, as well as many aggregates capable of further proliferation (specification, page 46, lines 11-14; emphasis in text).

The Applicants invention is thus not anticipated by Markowicz *et al.* because the dendritic cell cultures in Markowicz *et al.* are not an enriched and expanded population of proliferating dendritic cell precursors.

Markowicz *et al.* also fails to anticipate independent claim 101, as was implicitly acknowledged by the Examiner by failing to reject this claim. Indeed, as described above, Markowicz *et al.* fails to disclose or suggest an enriched and expanded population of processed antigen presenting dendritic cell precursors according to the instant invention. Instead, Markowicz *et al.* teaches away from the instant invention by stating that "...GM-CSF does not cause DCs to divide and proliferate." Because claims 89 and 94 depend directly from independent claim 101 (and claim 84, wherein it depends on claim 101), and thus contain all the limitations thereof, Applicants submit that none of these claims are anticipated over Markowicz *et al.* Applicants respectfully believe rejection of these claims was made in error, and request that this rejection be reconsidered and withdrawn.

Thus, Applicants submit that claims 82-85, 89, 95-97, as well as claims 89, 94, 101, as amended, and new claim 102, satisfy the requirements of 35 C.F.R. § 102.

Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

III. Conclusion

In view of the foregoing amendments and remarks, Applicants respectfully submit that this application is now in condition for immediate allowance. If a telephone interview would advance prosecution of the application, the Examiner is invited to call the undersigned at the number listed below.

A Petition for a four (4) month Extension of Time under 37 C.F.R. § 1.136(a) is filed concurrently herewith, which extends the response period from 03 September 2001 to 03 January 2002. The Petition further authorizes the PTO to charge the four month extension fee of $720 to our Deposit Account No. 08-0219, which reflects Applicants' Small Entity Status. If there are any other fees due in connection with the filing of the response, please charge the fees to our Deposit Account No. 08-0219. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above or in the Petition filed concurrently herewith, such an extension is requested and the fee should be charged to our Deposit Account. Also, please charge any fees underpaid or credit any fees overpaid to the same Deposit Account.

Respectfully submitted,

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# APPENDIX A

## (PENDING CLAIMS 82, 84, 85, 89, 94-97, 99, 101, AND 102 – CLEAN VERSION)

82. An *in vitro* composition comprising an enriched and expanded population of proliferating dendritic cell precursors.

84. (Twice Amended) The composition of dendritic cell precursors according to either claims 82 or 101, wherein the dendritic cell precursors are human.

85. (Amended) The composition of dendritic cell precursors according to claim 84, wherein the dendritic cell precursors are obtained from blood.

89. (Twice Amended) The composition according to claim 101, wherein the antigen is a microorganism.

94. (Twice Amended) The composition according to claim 101, wherein the antigen is a mycotuberculosis bacteria.

95. (Amended) The composition according to claim 94, wherein the mycobacteria tuberculosis bacteria is BCG.

96. A pharmaceutical composition comprising a therapeutic amount of an enriched and expanded population of human proliferating dendritic cell precursors and a pharmacologically acceptable carrier.

97. (Amended) The composition according to claim 96, wherein the dendritic cell precursors are antigen-activated.

99. (Amended) The pharmaceutical composition according to claim 97, wherein the antigen-activated dendritic cells express an amount of modified antigen to provide between about 1 to 100 micrograms of modified antigen in said pharmaceutical composition.

101. (Twice Amended) An *in vitro* composition comprising a population of antigen-activated dendritic cell precursors, wherein said antigen-activated dendritic cell precursors present processed antigen derived from an enriched and expanded population of proliferating dendritic cell precursors, which were contacted *in vitro*, in the presence of GM-CSF, with antigen for sufficient time for said proliferating dendritic cell precursors to process and present said antigen.

~~102~~ 103. (New) The pharmaceutical composition according to claim 97, wherein the pharmaceutical composition comprises from about $1 \times 10^6$ to $1 \times 10^7$ antigen-activated dendritic cell precursors.

# APPENDIX B

## (AMENDED CLAIMS 84, 85, 89, 94, 95, 97, 99 AND 101, AND NEW CLAIM 102 – MARKED UP VERSION)

84. (Twice Amended) The composition of dendritic cell precursors according to either claims 82 or 101, wherein the dendritic cell precursors are human.

85. (Amended) The composition of dendritic cell precursors according to claim 84, wherein the dendritic cell precursors are obtained from blood.

89. (Twice Amended) The composition according to claim 101, wherein the antigen is a microorganism.

94. (Twice Amended) The composition according to claim 101, wherein the antigen is a mycotuberculosis bacteria.

95. (Amended) The composition according to claim 94, wherein the mycobacteria tuberculosis bacteria is BCG.

97. (Amended) The composition according to claim 96, wherein the dendritic [cells] <u>cell precursors</u> are antigen-activated.

99. (Amended) The pharmaceutical composition according to claim 97, wherein the antigen-activated dendritic cells express an amount of modified antigen to provide between about 1 to 100 micrograms of modified antigen [per dose] <u>in said pharmaceutical composition</u>.

101. (Twice Amended) An *in vitro* composition comprising [an enriched and expanded] a population of [processed antigen presenting] antigen-activated dendritic cell precursors, wherein said antigen-activated dendritic cell precursors present processed antigen derived from [said] an enriched and expanded population of proliferating dendritic cell precursors, which were contacted *in vitro*, in the presence of GM-CSF, with antigen for sufficient time for said proliferating dendritic cell precursors to process and present said [processed] antigen.

Please add new claim 102:

102. (New) The pharmaceutical composition according to claim 97, wherein the pharmaceutical composition comprises from about $1 \times 10^6$ to $1 \times 10^7$ antigen-activated dendritic cell precursors.

# APPENDIX C

**Hunt *et al.* (1992) *Science* 255:1261-1263.**